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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Andrew J. Murphy, et al.
U.S. Serial No.:	Not yet known
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Title:	Methods of Modifying Eukaryotic Cells
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July 21, 2003

Commissioner for Patents
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

PRELIMINARY AMENDMENT

This Preliminary Amendment is being filed in connection with the divisional application filed concurrently herewith. Prior to examination of the subject application on the merits, Applicants respectfully request entry and consideration of the following amendments to the claims and specification.

Amendments to the Specification begin on page 2 of this paper.

Listing of the Claims begins on page 4 of this paper.

Fees begins on page 13 of this paper.

On page 1, please amend the paragraph beginning on line 2 as follows:

This application is a divisional of U.S. Serial No. 09/784,859, filed February 16, 2001, now allowed, which is a continuation-in-part of ~~claims priority to United States Patent Utility Application~~ U.S. Serial No. 09/732,234, filed December 7, 2000, now issued as U.S. Patent No. 6,585,251, which claims ~~priority to United States Provisional Patent Application~~ the benefit of U.S. Serial No. 60/244,665, filed October 31, 2000, now abandoned, each of which is incorporated by reference herein. Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

On page 16, please amend the paragraph beginning on line 24 as follows:

Figure 3A-3D (SEQ ID NOS: 5 and 6)**:** Sequence of the mouse OCR10 cDNA, homology box 1 (hb1), homology box 2 (hb2), and TaqMan® probes and primers used in a quantitative PCR assay to detect modification of allele (MOA) in ES cells targeted using the mOCR10 LTVEC.

hb1: base pairs 1 to 211
hb2: base pairs 1586 to 1801

TaqMan® probe and corresponding PCR primer set derived from mOCR10 exon 3:
TaqMan® probe: nucleotides 413 to 439 - upper strand
Primer ex3-5′: nucleotides 390 to 410 - upper strand
Primer ex3-3′: nucleotides 445 to 461 - lower strand

TaqMan® probe and corresponding PCR primer set derived from mOCR10 exon 4:
TaqMan® probe: nucleotides 608 to 639 - upper strand
Primer ex4-5′: nucleotides 586 to 605 - upper strand
Primer ex4-3′: nucleotides 642 to 662 - lower strand

On page 33 please amend the paragraph beginning on line 24, line 1, as follows:

(a) OCR10.RAA (5'-AGCTACCAGCTGCAGATGCGGGCAG -3' [SEQ ID NO: 1]) and OCR10.PVIrc (5'-CTCCCCAGCCTGGGTCTGAAAGATGACG-3' [SEQ ID NO: 2]) which amplifies a 102 bp DNA; and

(b) OCR10.TDY (5'-GACCTCACTTGCTACACTGACTAC-3' [SEQ ID NO: 3]) and OCR10.QETrc (5'-ACTTGTGTAGGCTGCAGAAGGTCTCTTG-3' [SEQ ID NO: 4]) which amplifies a 1500 bp DNA.

On page 34, please amend the paragraph beginning on line 21, as follows:

To construct the mOCR10 LTVEC, first a donor fragment was generated consisting of a mOCR10 homology box 1 (hb1) attached upstream from the LacZ gene in the modification cassette and a mOCR10 homology box 2 (hb2) attached downstream of the neo-PGK polyA sequence in the modification cassette (Figure 2), using standard recombinant genetic engineering technology. Homology box 1 (hb1) consists of 211 bp of untranslated sequence immediately upstream of the initiating methionine of the mOCR10 open reading frame (mOCR10 ORF) (Figure 3A-3D [SEQ ID NOS: 5 and 6]). Homology box 2 (hb2) consists of last 216 bp of the mOCR10 ORF, ending at the stop codon (Figure 3A-3D [SEQ ID NOS: 5 and 6]).

On page 34, please amend the paragraph beginning on line 6, as follows:

To identify ES cells in which one of the two endogenous mOCR10 genes had been replaced by the modification cassette sequence, DNA from individual ES cell clones was analyzed by quantitative PCR using standard TaqMan® methodology as described (Applied Biosystems, TaqMan® Universal PCR Master Mix, catalog number P/N 4304437; see also http://www.pebiodocs.com/pebiodocs/04304449.pdf). The primers and TaqMan® probes used are as described in Figure 3A-3D (SEQ ID NOS: 5 and 6) . A total of 69 independent ES cells clones where screened and 3 were identified as positive, i.e. as clones in which one of the endogenous mOCR10 coding sequence had been replaced by the modification cassette described above.